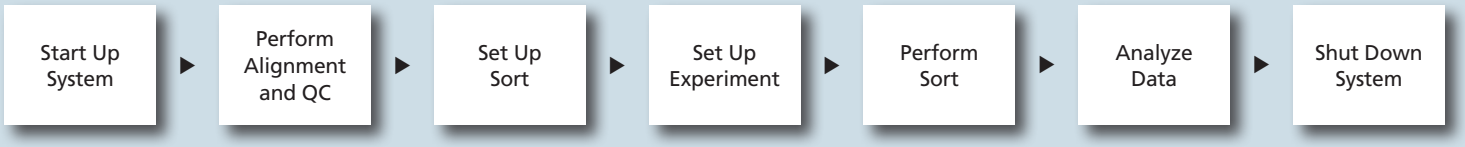
Getting Started Guide for Using BD FACSJazz™ Cell Sorters with BD FACS™ Sortware Sorter Software

This guide contains instructions for using BD FACS™ Sortware sorter software v1.X with BD FACSJazz™ cell sorters.

Workflow Overview

The following figure shows the daily workflow steps.



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Start Up the System

1. Turn on the cytometer and the computers.

    a. Turn on the main power.
        - Two connection confirmation beeps sound when the server has connected with the instrument. Three repeating beeps sound if the server is unable to connect to the instrument.
        - Allow 30 minutes for lasers to fully warm up before recording QC data.

    b. Turn on the BD FACS Sortware computer, if necessary.

2. Start BD FACS Sortware software.

    a. Log in to the BD FACS Sortware computer, if necessary.

    b. Double-click the BD FACS Sortware icon on the desktop to start the software.
        - Verify that the BD FACS Sortware workspace, the SortView pane, and the Pressure Control pane have opened.

    c. Restore or create a workspace for alignment and QC.
        - A QC workspace contains cytometer settings, sort settings, plots, and gates used for alignment and sort setup.

3. Prepare the fluidics.

    a. Fill the sheath tank and empty the waste tank.
        - Typical starting volume for daily use is 5 liters. This is based on an average usage of 0.5 liter per hour, plus 1 liter additional volume to account for startup rinsing and drop stability. The maximum tank volume is 7 liters.
        - Bleach may be added to the waste tank so that the end-of-day concentration of bleach is 10%. Do not let undiluted bleach sit in the waste tank while the instrument is not in use.

    b. Reattach the sheath filter, if necessary.

    c. Turn on the vacuum and air supply sources.

    d. Turn the AIR switch to on.

e. Connect the air, vacuum, and fluidic lines to the sheath and waste tank.
f. Verify that the sheath tank is pressurized to ~27 psi, and the waste tank is between 5" and 15" Hg vacuum.

4 If performing a **dry startup**, clean and attach the nozzle tip.

a. Sonicate the nozzle tip in a tube of distilled water for one minute.
   - If sonicating the tip in ethanol or detergent, rinse the tip with distilled water before installing on the instrument.
b. Use a syringe to flush the nozzle tip with filtered deionized water.
c. Place the flush bucket under the nozzle assembly, if necessary.
d. Install the nozzle tip onto the nozzle assembly.

4 If performing a **wet startup**, remove the debubble reservoir and leave the flush bucket under the nozzle assembly.

5 Flush the system to remove air from the sheath filter.

a. Click **Rinse** to begin flushing the system.
b. Tap the sheath filter gently to dislodge any air bubbles.
c. Click **Rinse** to stop flushing the system.

6 Remove air from the sample line and nozzle.

a. Click **Stream** to start the stream.
b. Click **Backflush** and verify that sheath fluid is dripping from the sample line.
c. After about 20 seconds, click **Backflush** and then click **Stream** to stop the stream.
d. Install the debubble reservoir filled with filtered sheath fluid so that the nozzle tip is submerged.
e. Click **Purge** to remove air from the nozzle.
f. Click **Pulse** to help dislodge bubbles.
   - If performing the dry startup, allow the initial air bubble from the nozzle to pass the y-fitting before clicking **Pulse.**
g. (Optional) If bubbles are still present after pulsing:
   - Use the "nozzle prime" technique to dislodge bubbles: While in purge mode, remove the debubble reservoir to introduce a small amount of air into the nozzle. Then, refill the debubble reservoir and re-submerge the nozzle.
   - Or, stop the PURGE and fill the debubble reservoir with 70% ethanol and click **Purge** to fill the nozzle.
     Click **Pulse** to help dislodge bubbles. Stop the purge and refill the debubble reservoir with sheath fluid. Purge with sheath fluid to clear away the ethanol.
h. Click **Purge** once air has been removed from the nozzle.

7 Start the stream.

a. Install the two-tube sort device with 50-mL conical, and put the tray in the Safe position.
b. Open the deflection plates before removing the flush bucket to reduce the risk of getting them wet.
    - (Optional) Before removing the flush bucket, remove and clean the deflection plates. Dry the plates thoroughly before replacing.
c. Click **Stream** to start the stream.
d. Remove the debubble reservoir.
e. Remove any excess fluid from the nozzle tip using a tissue or cotton applicator.

Perform Alignment and QC

1. Align the stream to the pinholes, the stream drain, and the BD FACS™ Accudrop laser.

    a. Open the nozzle access door and close the sort chamber door, if necessary.
    b. Press Illum to turn on the Accudrop laser.
    c. Remove the flush bucket from under the nozzle, if necessary.
    d. Use the three translational (horizontal, vertical and focus) silver knobs on the nozzle stage to align the upper section of the stream to the pinholes.
        - Usually, the vertical-axis knob should not be adjusted for daily use.
        - The stream should be centered on the pinholes with crisp edges on the sides of the stream.
        - The nozzle tip should be about one pinhole distance above the first pinhole.
    e. Use the two rotational (pitch and roll) black knobs on the nozzle stage to align the lower section of the stream with the stream drain and the Accudrop laser.
        - (Optional) Advanced users can use Test Streams to align the side streams to the Accudrop laser. The nozzle access door must be closed to charge the stream.
    f. Repeat steps 1d and 1e until the stream is centered and in focus over the pinholes and centered in the stream drain and Accudrop laser beam.
    g. Close the nozzle access door.

2. Optimize the fluorescence and side scatter parameters from the primary laser.

    a. Open the shutter for the primary laser.
    b. Verify that the nozzle access door is closed and activate the laser reset sensor.
    c. Run a sample tube of QC beads and adjust the sample offset to produce a narrow sample core.
        - Verify that the bead flash for the primary laser is aligned with the first pinhole, and adjust if necessary.
        - Verify that the trigger parameter is set to SSC or primary fluorescence.
    d. While looking at dot plots for primary fluorescence and SSC channels, make small adjustments to the stream alignment to obtain the brightest signal strength and lowest CV.
        - If CVs remain high or signal strength low, lower the Sample Offset and/or make small adjustments to the stream primary laser adjustment knob.

3. Optimize forward scatter (standard FSC).

    a. Examine the FSC signal.
        - With good consistent daily alignment, little or no adjustment is needed.
    b. If you need to optimize the FSC signal:
        - Lower the Sample Offset, being careful not to collapse the core stream.
        - Make small adjustments using the knobs on the forward scatter stage.

Perform Alignment and QC (Continued)

4. Align the remaining lasers, if present.

    a. Open the appropriate laser shutter.
        - Verify that the laser is aligned with the appropriate pinhole.
    b. Optimize the laser delay for each laser to achieve the brightest and tightest signal.
        - Typical values for laser delay for the second and third pinhole are 5.2 and 10.4, respectively.
        - Laser delays may be further optimized after laser adjustment in step 4c.
    c. Make small adjustments using the appropriate laser adjustment knob to obtain the brightest signal strength and lowest CV.

5. Enter values in the QC log.

    a. Adjust the voltages to place the fluorescence and scatter signals at their target values.
    b. Record the PMT voltage and rCV for each parameter in the QC log.
        - Optionally, QC may be performed by keeping the PMTV constant and recording the median signal strength and rCV for each parameter.
    c. Record a data file for QC.

Set Up to Sort

1. Optimize the frequency.

    a. Visualize the breakoff using the Drop Camera image.
        - The typical starting value for piezo amplitude is between 4 and 5.
        - If the drop position is off-screen, adjust the **Piezo Amplitude** settings.
        - If the piezo needs to be adjusted dramatically, or the drops are moving, check the sheath tank for leaks and the nozzle for air bubbles.

    b. Scan for the optimal frequency to find the shortest breakoff.
        - The typical starting value for the drop frequency is 39.00 kHz.

    c. Adjust the **Piezo Amplitude** to position the breakoff at the drop reference marker.

2. Optimize the drop phase.

    a. Verify that the two-tube holder is installed, with the waste conical in place, on the sort stage.

    b. Select **2 tube holder – 2 way sort** as the sort device in the **Sort Layout**.

    c. Click **Safe** in the **Sort Layout** to place the waste conical under the waste drain.

    d. Close the deflection plates then click **Plates** to charge them, if necessary.

    e. Click **Test Streams** in the **Sort Settings** pane to initiate stream deflection.
        - Confirm that the nozzle chamber door, the sort chamber door, and the plate access panel are closed.

    f. Click **Flash Charge** and adjust the **Piezo Amplitude** so that the side streams are maximally deflected with no fanning.
        - If you cannot get side streams to deflect without fanning, adjust the frequency and/or clean the deflection plates and repeat.
        - Verify that the breakoff is at the drop reference marker. If it is not, adjust the **Piezo Amplitude** to bring the breakoff to the marker and repeat the Flash Charge.

    g. Click **Short Flash** and adjust the **Piezo Amplitude** so that the side streams are maximally deflected with no fanning.

    h. Turn off the Short Flash and Flash Charge and adjust the stream focus to eliminate second drop deflection, if necessary.
        - The typical starting value for stream focus is 10 to 12%.

    i. Turn off the Test Streams.

3. Determine the drop delay using BD FACS™ Accudrop.

    a. Click **Safe** in the **Sort Layout** pane and verify that the waste flask is under the stream drain.

    b. Select **Accudrop Setup** as the sort device in the **Sort Layout**.

    c. Prepare and run a sample tube of BD FACS™ Accudrop beads and adjust the sample offset to produce an event rate near 2,000 events per second.

    d. Click **Accudrop** to place the Accudrop optical filter into position.

    e. Create a gate for the Accudrop beads, if necessary.

    f. Assign the gate for the Accudrop beads in the **Sort Layout** pane and click **Start** to begin sorting.

g. Adjust the drop delay until the left stream is as bright as possible and the center stream has almost no fluorescence.
h. Click **Accudrop** to place the neutral density filter into position

4. Align the sort streams to the sort device.

a. Select the desired sort device in the **Sort Layout**, and install the appropriate sort device on the sort tray.
b. Adjust the side streams and/or tray position so they are centered in the sort collection tube or well.

Set Up an Experiment (with Compensation)

1. Prepare the workspace.

    a. Open a new workspace or load configuration files.
        - If restoring a workspace, clear the **Fluidics Setup** checkbox to prevent overwriting the optimized daily sort settings.
    b. Verify the user preferences.
    c. Verify the following cytometer settings: trigger detector level and parameter, and the Lin or Log amplifier setting for each parameter, then enter the detector channel labels.
        - It is important to remember that you can sort only what you see, and you can exclude from your sort only what you see.
    d. Create and/or verify worksheet elements such as plots, statistics, and gate hierarchies, if necessary.

2. Adjust PMT detector voltages to optimize for the sample.

    a. Run an unstained sample tube, and verify the scatter detector voltages and trigger level, if necessary.
        - If you cannot determine the scatter voltages, use a single or fully stained control to help identify the population through gating.
    b. Run the single stained control sample tubes or the fully stained sample, and verify the fluorescence detector voltages.
        - PMT voltages for all detectors to be compensated must be adjusted before recording compensation controls. The Autocompensation tool will not process tubes with different detector voltages.

3. Record compensation controls.

    a. Load and run the unstained control sample tube.
    b. Change the save location and file name to reflect the sample type (for example, *unstained control*).
    c. Verify the recording rule settings, including: the event limit, stopping gate, and storage gate.
        - A minimum of 100 events in each gate is necessary per compensation control population to properly calculate compensation.
    d. Record a data file for the unstained control tube.
    e. Repeat steps 3a–3d for each of the single color control tubes.

4. Perform compensation.

    a. Add a new worksheet page.
    b. Create ADC data plots for each of the compensation control data files.
    c. Create Local gates for each compensation control population, and name them accordingly.
    d. Verify that **Cytometer** is the source in the **Compensation** pane.
    e. Select the parameters to be compensated using **Manage Parameters**.

f. Assign the positive and negative compensation control populations to the parameters for autocompensation.
g. Click **Calculate** to populate the compensation matrix.
h. (Optional) Select the **Visualize** box in the **Compensation** pane to view compensation applied to the ADC parameters.
    - Compensation visualized using the ADC parameters can be displayed in Logicle scaling, and the compensation values can be adjusted post-recording.

Perform a Sort

1. Create sort gates and worksheet elements.

    a. Load the sort sample and acquire some data.
    b. Create or modify plots, if necessary.
        - Verify that *DSP parameters are used to display compensated parameter data.
    c. Create or modify gates, if necessary.
        - Verify that *DSP parameters are used to define sort gates using compensated parameter data.
    d. Create or modify population hierarchies and/or statistics views, if necessary.
        - Verify the percentages of target populations and plan the sort accordingly.

2. Set up the Sort Layout.

    a. Select a sort device in the **Sort Layout** and install the sort device onto the sort tray, if you have not already done so.
    b. Verify that the side streams are aligned to the sort device, and adjust if necessary.
    c. Assign the sort populations to sort positions.
    d. Assign the sort limit for each sort position.
    e. Select the sort mode to be used during the sort.
    f. Save the **Sort Layout**.

3. Start the sort.

    a. Verify that the droplet position and breakoff either visually or by using the Flash Charge, before starting the sort.
        - If using the Flash Charge, remember to remove the sort device and replace with the waste flask while testing streams.
    b. Mix and or filter the sample, if necessary.
    c. Load and run the sort sample tube.
    d. Adjust the sample offset to achieve the desired acquisition event rate.
        - In general, the higher the event rate the lower the sort efficiency. We recommend that the event rate not exceed 1/4 of the drop frequency.
    e. Click **Sort Ready** in the **Sort Layout** pane to place the sort device in the starting sort position.
    f. Start the sort.

4. Monitor the sort.

    a. Monitor the acquisition event rate and acquisition efficiency.
        - Adjust the sample offset, if necessary.
    b. Monitor the sort rates, sort abort rates, and sort efficiency.
    c. Monitor the sort streams and minimize fanning, if necessary.
        - Mitigate fanning by adjusting the piezo amplitude, event rate, sort mode, or by filtering the sample.
        - Fanning due to cell size and shape may necessitate using a larger nozzle for sorting. Generally, we recommend using a nozzle at least five times larger than the cells in the sample.

5 Complete the sort.

a. Verify that the sort has completed, or stop the sort manually.
b. Stop and unload the sample.
c. Click **Eject** in the **Sort Layout** pane, if necessary, and remove the sort device.
d. (Optional) Preview, print, and/or save the sort report.
    - The sort report can be reset by clicking **Reset** in the **Sort Layout** or by changing the sort device.

Analyze Data

1. Record post-sort data files.

    a. Transfer a portion of the sort sample to a 12 x 75-mm polypropylene sample tube.
    b. Backflush the sample line between samples to prevent cross-contamination.
        - If desired, run a clean sample tube of filtered water between samples to check for residual sample in the sample lines.
    c. Run and record your post-sort samples.

2. Verify the analysis.

    a. If you have created an Analysis Template for the experiment, restore the Analysis Template for the post-sort data file. Or, add new worksheet pages and create plots, population hierarchy, and/or statistics view for the post-sort data files.
    b. Create, modify, and/or localize gates for analysis, if necessary.
    c. (Optional) Select the **Visualize** box in the **Compensation** pane to view compensation applied to the ADC parameters.
        - Compensation visualized using the ADC parameters can be displayed in Logicle scaling, and the compensation values can be adjusted post-recording.
        - Compensation applied to the DSP parameters cannot be adjusted after recording.
    d. Check the sort purity using post-sort data files.
        - Sort gates may need to be localized and modified to analyze post-sort purity due to wider distribution of target populations after sorting.
        - If sort purity was lower than expected, modify the sort mode, sort gates, and/or include a doublet discrimination gate, and perform the sort again.

3. Save, print, or export the results.

    a. Save the Workspace.
    b. Save any additional configuration files, if necessary, including Cytometer Settings, Sort Layout, Analysis Template, and Compensation.
    c. Save and/or print the worksheet report as a PDF file.
    d. (Optional) Export data files as CSV files or in BD FACSDiva™ software compatible format.

Shut Down the System

1. Clean the sample line.

    a. Load and run a sample tube of 10% bleach for 5 minutes.
    b. Load and run a sample tube of filtered distilled water for 5 minutes.

2. Perform a **wet shutdown.**

    a. Leave the tube of water on the sample port.
    b. Place the flush bucket under the nozzle.
    c. Fill the debubble reservoir with filtered distilled water and install it on top of the flush bucket.
    d. Verify that the nozzle tip is submerged.
    e. Stop the stream and PURGE for 1 minute to fill the nozzle with water.
    f. Remove the air, vacuum, and fluidic lines from the sheath and waste tanks.
        - DO NOT connect the fluidic and air lines together during a wet shutdown.
    g. Empty the sheath and waste tanks.
        - If bleach was not added now before sampling, bleach may be added to the waste tank to a concentration of 10% and allowed to sit for 15 minutes before disposal for decontamination, if necessary.

2. Perform a **dry shutdown.**

    a. Remove the tube of water from the sample port.
    b. Place the flush bucket under the nozzle.
    c. Stop the stream, and remove the air, vacuum, and fluidic lines from the sheath and waste tanks.
    d. Empty the sheath tank and fill it with 0.5–1.0 L of distilled water.
    e. Empty the waste tank.
        - Bleach may be added to the waste tank to a concentration of 10% and allowed to sit for 15 minutes before disposal for decontamination, if necessary.
    f. Reconnect the air, vacuum, and fluidic lines to the sheath and waste tank and verify that the vacuum and pressure have been applied to the tanks.
    g. Bypass the sheath filter.
        - (Optional) Bypass the sheath filter one minute after beginning step 2i so that the sheath filter is stored in water instead of sheath.
    h. Click **Rinse** and then **Backflush** to run water through all the lines.
    i. When air starts to run through the system, click **Rinse** and then **Backflush** to stop the stream.
    j. Remove the air and sheath lines from the sheath tank and connect them to each other.
        - Do not connect the air and sheath lines to each other if the sheath filter is still in place.
    k. Click **Rinse** and **Backflush** to remove any residual water, then click **Rinse** and **Backflush** to stop the flow, then remove the nozzle tip and place it in a storage vessel.
    l. Click **Rinse** and **Backflush** to dry the fluidic lines, and stop fluidics when system is completely dry.

m. Empty the sheath and waste tanks.
   - Bleach may be added to the waste tank to a concentration of 10% and allowed to sit for 15 minutes before disposal for decontamination, if necessary.

3. Clean the sort chamber.

   a. Verify that the power to the deflection plates is turned off.
   b. Remove the plate access panel and clean the deflection plates.
   c. Clean and decontaminate any spills in the sort chamber or sample port area.
   d. Clean any area of salt buildup and check for leaks.

4. Turn off the power.

   a. Export files to secondary storage device, if needed.
   b. Save the workspace, if needed.
   c. Save Cytometer Settings and Sort Layout, if needed.
   d. In BD FACS Sortware, select **Cytometer** > **Shutdown Cytometer**.
      - The Shutdown Cytometer command disconnects and shuts down the server.
      - (Optional): If the instrument needs to be used after Cytometer > Shutdown Cytometer was selected, switch the cytometer power off, then on again to restart and reconnect the cytometer server.
      - Turn the AIR switch to Off.
   e. Turn off the vacuum and the air supply.
   f. Turn off the BD FACS Sortware computer.
   g. Turn off the cytometer power.